2041-Pos Board B811
Homo-FRET Studies of the Signal Recognition Particle Protein FFH by Multiparameter Fluorescence Detection (MFD) and Filtered Fluorescence Correlation Spectroscopy (FFCs)
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The group of Wolfgang Wintermeyer has previously performed experiments on a protein Ffh from the Signal Recognition Particle (SRP) (Buskiewicz et al., JMB 2005). Using multiple double cystein mutants and labelling with Bodipy-FL they measured the changes of anisotropy for single labeled and double labeled Ffh in presence and absence of 4.5 sRNA in bulk. From these measurements, the extracted distances were compared to three different existing crystal structures of Ffh (Keenan et al., Cell 1998; Rosendal et al., PNAS 2003). The distances were in agreement with A/A structure of *T. aquaticus* (Keenan et all., Cell 1998).
We use two recently developed approaches to study dynamics of Homo-FRET samples at single molecule level: Anisotropy photon distribution analysis (aPDA) and filtered fluorescence correlation spectroscopy. Anisotropy PDA is an extension of existing method (Kalinin et al., JPC 2007), and allows us to separate different populations by differences in anisotropy. Filtered FCS is a new and powerful modification of the standard FCS approach. Using the information of the fluorescence lifetime and anisotropy decays, we filter the signal and weight the contribution of each single photon to the corresponding population of the probe. The weighted time dependent signal is then correlated like a standard FCS, allowing for a sub-microsecond resolution. As the result we obtain auto- and cross-correlation curves of which cross-correlation curve allows for to extract species selected interconversion rates.
Applying both tools on the homo-FRET labeled Ffh will allow us to determine the conformational space of the protein and the corresponding conformational kinetic in a single molecule experiment free in solution.

2042-Pos Board B812
Brightness Characterization of Cytoplasmic & Membrane-Bound Protein Mixtures by Z-Scan Fluorescence Fluctuation Spectroscopy
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Fluorescence fluctuation spectroscopy (FFS) has been employed to quantify the stoichiometry of soluble proteins in the nucleus and cytoplasm of mammalian cells. However, some proteins have both cytoplasmic and membrane-bound components which render traditional FFS methods inapplicable due to coexcitation of the spatially distributed protein mixture. In this work, we apply z-scan FFS to characterize the brightness of both the membrane-bound and cytoplasmic populations of a protein. Experimentally, we choose HRas-EGFP as our membrane-bound/cytoplasmic model system. We use a combination of stationary FFS measurements to determine the brightness of membrane-bound and cytoplasmic HRas-EGFP and z-scan intensity profiles to correct for brightness bias due to thin layer geometry and coexcitation of membrane and cytoplasmic layers. Our data show HRas-EGFP to be monomeric at both the upper and lower plasma membrane and also in the cytoplasm. We further examine the brightness statistics of proteins by comparing the experimental brightness distribution of a membrane-bound protein system with that of a soluble protein system. This work is extended by creating a dimeric membrane protein system in order to establish a model for calibrating brightness and stoichiometry. The z-scan FFS measurement technique along with a reliable monomer/dimer calibration system will be important for future membrane protein stoichiometry studies. This research was supported by grants from the National Institutes of Health (GM64589) and the National Science Foundation (PHY 0346782).

2043-Pos Board B813
Quantifying Ternary Protein Expression Fractions using Three-Channel Fluorescence Fluctuation Spectroscopy
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Fluorescence studies of cellular protein-protein interactions commonly employ transient transfection to express proteins carrying distinct fluorescent labels. Because transiently transfected cells differ significantly in their expression level, the concentration ratio of the expressed proteins varies, which in turn influences the measured fluorescence signal. Knowledge of the statistics of protein expression ratios, which currently is not well understood, is of considerable interest both from a fundamental point of view and for cellular fluorescence studies. Despite the perceived randomness of transient transfection, we recently succeeded in developing a model that describes the distribution and the average of the protein expression ratio for the coexpression of two proteins. Here we extend our previous work to the simultaneous coexpression of three proteins, where each protein species is labeled with a distinctly colored fluorescent protein. All protein species are simultaneously excited using two-photon absorption. The fluorescence of the protein mixture is split according to color into three detection channels. Brightness measurements of the individual proteins combined with intensity ratio data between detection channels is used to identify the protein expression ratios of all three proteins. We examine the relationship between DNA-plasmid fractions and protein expression fractions. In addition, we quantify the cell-to-cell scatter in the protein expression ratios for different transfection protocols in order to identify the best conditions for three-color fluorescence fluctuation experiments. This work was supported by grants from the National Institutes of Health (GM64589) and the National Science Foundation (PHY 0346782).

2044-Pos Board B814
Conformational Fluctuations of Chromosomal DNA in E-Coli
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We measured the conformational fluctuations of DNA in *E. Coli in* vivo using fluorescence correlation spectroscopy (FCS). The chromosomal DNA was randomly decorated with a cell-permeable intercalating dye. Conformational fluctuations of the DNA move the fluorophores stochastically into the diffraction-limited excitation volume of a focused laser beam. The time correlation function of the fluorescence intensity reflects the underlying dynamics of the DNA on length scales down to ~200 nm. A comparison between live cells and dead yet structurally intact cells shows identical fluctuation spectra for short time scales, yet substantial differences for frequencies below 100 Hz. Live cells show much stronger fluctuations in this regime. This observation points to the crucial importance of active molecular motor action, as opposed to passive thermal noise, in driving larger conformational fluctuations in the chromosomal DNA, in particular on length scales exceeding ~500 nm.

2045-Pos Board B815
Time-Shifted Fluorescence Cumulant Analysis in Fluorescent Fluctuation Spectroscopy
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Brightness analysis of fluorescence fluctuation spectroscopy (FFS) data has been developed as an analytical and experimental tool for studying protein interactions quantitatively and non-invasively in living cells. Real photon count detectors introduce dead-time and afterpulsing effects, which alter the measured photon count statistics. Careful consideration of these detector artifacts in the analysis of FFS experiments is crucial to avoid misinterpretation of FFS experimental data and is especially important for cell based studies, where large differences in concentration introduce various degrees of dead-time and afterpulsing bias. Even with careful and lengthy characterization of the detector, the correction process becomes unstable for large bias values. Brightness analysis based on the photon count distribution or its moments is strongly affected by dead-time and afterpulsing. Here, we introduce an alternative brightness analysis method called time-shifted fluorescent cumulant analysis. The new analysis leads to significantly reduced detector artifacts than the previously used conventional methods, and therefore is an attractive method for cell-based FFS studies. In this study, we demonstrate the capabilities of time-shifted fluorescence cumulant analysis and compare it with conventional brightness analysis methods. This work was supported by grants from the National Institutes of Health (GM64589) and the National Science Foundation (PHY 0346782).

2046-Pos Board B816
Förster Resonance Energy Transfer as a Probe of Membrane Protein Folding
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The molecular mechanisms of the folding reaction of an integral membrane protein, outer membrane protein A (OmpA), are investigated with Förster resonance energy transfer (FRET). Here, we report six mutants of the transmembrane portion of OmpA with intrinsic donor (tryptophan) and extrinsic acceptor (IAEDANS) in various locations of the protein to probe the evolution of distances during insertion and folding into lipid bilayers. Control experiments of donor-only and acceptor-only OmpA mutants have also been performed to determine the Förster distances (Ro) in the folded and unfolded states; the Ro values in both protein conformations are 21 Å. The FRET efficiencies